Cover Illustration

Investigator puts the finishing touches on a molecular model of a possible deoxyribonucleic acid structure (DNA).

HIGHLIGHTS OF HEART PROGRESS—1960

Items of Interest on Research Studies Conducted and Supported by the National Heart Institute

Most of these items were prepared for submission at congressional hearings on appropriations for fiscal year 1962

U S DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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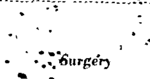
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ATHEROSCLEROSIS AND CORONARY HEART DISEASE

MER-29 Lowers Cholesterol by Blockade of Final Step in Its Synthesis by Liver

Studies by scientists at the National Institutes of Health have pinpointed the mechanism by which MER-29 lowers serum cholesterol levels By blocking the conversion of desmosterol to cholesterol, the drug inhibits cholesterol synthesis by the liver at the very last step of the complex sequence of reactions by which the cholesterol molecule is formed.

These studies were made by Drs Joel Avigan and Daniel Steinberg, of the National Heart Institute's Laboratory of Cellular Physiology and Metabolism, and Mr Malcolm J. Thompson and Dr. Erich Mosettig of the National Institute of Arthritis and Metabolic Diseases. Their findings are reported in *Progress Against Cardiovascular Disease* (2 525-530)

The scientists observed that the sterol material in the livers of rats treated with MER-29 had properties that differed subtly, but definitely, from those of cholesterol, the major sterol of normal livers. Careful analysis revealed the presence of large amounts of desmosterol, a compound identical to cholesterol except for two missing hydrogen atoms at one point in the molecule

When they injected radioactive acetate, a cholesterol precursor, into animals pretreated with MER-29, they found that most of the radioactivity accumulated in desmosterol rather than in cholesterol Clinical studies also showed that desmosterol accumulated in high concentrations in the serum of patients receiving the drug

These and other findings indicated that MER-29 lowers serum cholesterol by inhibiting the desmosterol-cholesterol conversion. They also help to clarify the process of normal cholesterol biosynthesis in man by providing factual evidence that desmosterol is an intermediate in this process

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co [illegible]

m: [illegible] from fats, carbohydrates, or proteins—all of which can give rise to acetate, the primary building block. Thus the possibility of lowering serum cholesterol levels by the use of drugs which inhibit the body's own production of cholesterol has attracted increasing attention during the past few years MER-29 appears to be the most promising of the compounds developed thus far

The synthesis of MER-29 was announced in 1958 by Frank Palopoli and co-workers at the William S. Merrell Company, Cincinnati, and its cholesterol-lowering effects in rats were announced in 1959 by Thomas Blohm and Robert Mackenzie of the same company. The drug, also called "Triparanol," is marketed by the William S Merrell Company, who also made the drug available for clinical research

The NIH clinical studies showed that MER-29 reduced serum cholesterol levels in patients without producing any observed toxic effects. However, the fall in serum cholesterol was accompanied by a rise in serum desmosterol, so that total serum sterol levels were not greatly changed, and were actually increased in some patients. The investigators suggest that the biologic role of desmosterol warrants more thorough investigation, since information thus gained might be pertinent to *the safe long-term use of drugs, such as MER-29, which might lead to* the pile-up of compounds normally present in the body only in very *low concentrations.*

Grantee Study Evaluates Prognosis of Victims of First Heart Attacks

An NIH grant-aided study of survival and factors affecting survival after the acute phase of first heart attacks (myocardial infarction) has shown that more than half of the 224 patients studied survived for five years or more The five-year outlook was brighter for younger patients than for older ones, was brighter for men than for women, and was brightest for patients able to resume normal activity after their attacks.

The prognosis of patients surviving for more than one month after their first clinically diagnosed heart attack was evaluated by Drs. J. L Juergens, J. E. Edwards, R. W. P Achor, and H. B. Burchell, of the Mayo Clinic, Rochester, Minnesota The scientists determined the rates of survival for 224 patients three, five, and ten years after the initial attack. They also evaluated statistically the effects on survival of such factors as age at onset, sex, pre-existing angina or hypertension; congestive heart failure developing subsequent to the attack; occupation (active or sedentary); and degree of physical disability resulting from the attack Their findings are reported in the *A M.A Archives of Internal Medicine* (*105.* 444-450)

The average age at time of onset was 62 years (60 years for men, 67 5 years for women) Follow-up of these patients showed that 68.8% survived for three years or more after their attacks, 55 4% for five years or more, and 29.2% for ten years or more

Not surprisingly, five-year survival rates were highest among those suffering attacks in their forties or fifties (78 6% and 62 5%), declined moderately among those having attacks during their sixties (to 56 6%),

then more sharply among those having attacks in their seventies or eighties (to 25.9% and 20%, respectively) Also no surprise was the finding that pre-existing angina or hypertension, or congestive heart failure developing after the attack, made the five-year prospect bleaker—by about 25% on the average.

The study also indicated that five-year survival rates were not significantly affected by the degree of physical activity involved in the occupations of male patients, were considerably higher for men than for women; and highest for those patients who were able to resume normal activity after their attacks The better prognosis enjoyed by the men could not be fully accounted for either by age differences at time of onset or by any other factors observed in the course of the study. The excellent five-year survival rate (73.2%) of patients who were able to resume normal activity after their attacks has also been observed in other studies, and appears to be the best index of a good prognosis

Necropsy studies on 79 patients who survived their initial attacks but died later revealed that 82% subsequently died from cardiovascular disease. Of these, 34% succumbed to recurrent myocardial infarction

Hyaluronidase Reduces Edema in Ischemic Heart Muscle

The edematous fluid accumulations in heart muscle which follow coronary artery ligation in dogs, are markedly reduced by hyaluronidase injections, report Drs. J Martins de Oliveira, M N. Levy, and Mr M I Schock, of St Vincent Charity Hospital in Cleveland Their NHI-supported studies are reported in the *American Heart Journal* (60 106–109)

Dr Martins de Oliveira and co-workers tied off a branch of the left coronary artery in 14 dogs to produce infarction and edema of the posterior papillary muscle (PPM). Eight of these dogs received hyaluronidase intravenously immediately after ligation and at hourly intervals for 5 hours, whereas the remaining 6 dogs received placebo injections of saline solution Six non-ligated dogs served as normal controls.

Specimens of the PPM and of anterior superior left ventricular tissue (ASLV) not affected by coronary ligation, were taken from the animals at sacrifice 5 hours after ligation. Differences in tissue water content, measured by [illegible] the tissues in an oven, [illegible] formation

From these studies the scientists found that PPM water content averaged 77 48 percent in the hyaluronidase-treated group, but increased to an average of 79 92 percent in dogs that received only placebo injections ASLV values in both of these groups, 76 39 and 76 21 percent respectively, approximated normal control values obtained from

non-ligated dogs The differences between normal and ischemic myocardium in the experimental groups are statistically significant, report the investigators.

"In the present study, hyaluronidase significantly reduced the water content of the ischemic myocardial areas, bringing it to values close to those of the normal regions," conclude the Cleveland scientists. However, they state, "The practical application of our experiments is still uncertain, since it remains to be proved whether or not it is beneficial to reduce edema in an ischemic myocardium. It may be postulated, however, that the increased tissue pressure caused by this edema may exert a compressive action upon the collateral vessels, thereby reducing still more the flow of blood to the damaged areas Further investigations are now being carried out in an effort to answer this question"

Hyaluronidase, or "spreading factor," is a mucolytic enzyme used by physicians to promote diffusion and absorption of drugs injected subcutaneously. Recent findings by Hungarian scientists, who demonstrated that intravenous hyaluronidase produces a faster diffusion of fluids through extracellular spaces and enhances lymphatic drainage, provided the basis for using hyaluronidase in the present study

Heart Attacks Treated by Coronary Injections of Clot-Dissolving Enzyme

A technique using a cardiac catheter to inject the clot-dissolving enzyme fibrinolysin into the coronary arteries has been used by NHI grantees in the treatment of eight patients with myocardial infarction. The procedure, which was well tolerated by the patients, appears to possess two important advantages over oral or intravenous means of administering fibrinolytic therapy in such cases It provides high concentrations of clot-lysing enzymes at the site of the clot without unduly elevating whole-system concentrations; and it allows intermittent perfusion, a procedure that appears to be more effective in dissolving clots than is continuous perfusion.

These studies were conducted by Drs Robert J Boucek and William P. Murphy, Jr, of the University of Miami School of Medicine, Miami, Florida Their findings are reported in the *American Journal of Cardiology* (6 525-533)

The technique involves passing a cardiac catheter into the aorta via the brachial artery and, under fluoroscopic guidance, positioning the catheter tip at the root of that great vessel This brings the catheter orifice in line with the coronary branches, so that fibrinolysin injected through the catheter will be swept into these vessels with their blood supply. To assure that the fibrinolysin is delivered at the exact time that the coronaries are filling with blood, the injection is made by a small pump activated by the R wave of the electrocardiograph. Best

results were obtained in this study when injections were made during every fourth cardiac cycle.

The patients selected were treated within 4-12 hours after onset of the attack. Because of its reported enhancement of clot lysis, heparin (an anticoagulant) was perfused with the fibrinolysin (as Thrombolysin: Merck, Sharp, and Dohme)

The effectiveness of therapy was evaluated on the basis of the patients' clinical response, subsequent ECG data; and determinations of serum glutamic oxaloacetic transaminase (GOT), an enzyme released from injured heart tissue and which provides an index of the extent of heart damage. No attempt was made to evaluate clot lysis by angiology because it was felt that injections of radiopaque substances, which are somewhat toxic, might be harmful to heart tissue already hyperirritable

The evaluation, though limited by available methodology, suggested that most patients were benefited by the therapy. All had an unusually mild clinical course in the hospital All but two had serum GOT levels considerably lower than those of unperfused controls, indicative of less heart muscle damage. GOT levels were lowest in patients treated within four hours of the attack, highest in those treated 9-12 hours after This indicates that fibrinolytic therapy, to be effective, must be initiated quickly, since it appeared that heart damage became irreversible about four hours after myocardial infarction.

While this study does not offer conclusive proof that fibrinolytic therapy is beneficial in the treatment of myocardial infarction, the technique used may prove extremely useful for future studies in this important, rapidly developing research area

Androsterone Linked to High Serum Cholesterol of Thyroid Deficiency

Androsterone, an important end-product of the metabolism of certain male sex hormones (androgens), may be an important factor in the high serum cholesterol levels that usually accompany thyroid deficiency

NHI grant-aided studies on the role of thyroid hormones in steroid metabolism have shown that androsterone levels in the blood are below normal in patients with thyroid deficiency and high in those with hyperthyroidism The studies also showed that injections of androsterone produced a sharp and significant fall in the serum cholesterol levels of normal subjects and those with hypercholesterolemia These findings suggest that androsterone, a metabolite of certain androgens, may be an important determiner of serum cholesterol levels, since these are usually elevated in hypothyroid patients and low in those with hyperthyroidism

These findings are summarized in *Annals of the New York Academy of Sciences* (*86*: 605–611) by Drs. T. F. Gallagher, Leon Hellman, H L. Bradlow, B. Zumoff, and D. K. Fukushima, of the Sloan-Kettering Institute for Cancer Research, New York.

Androsterone is one of two major end-products from the metabolism of testosterone and certain other androgens; the other, etiocholanone, is structurally similar but differs sharply from androsterone in its biological activities The scientists found that, in euthyroid patients (normal thyroid function), the ratio of androsterone to etiocholanone was 40.60. However, the androsterone fraction was only 15% or even less in patients with hypothyroidism from myxedema; conversely, it was 50% or higher in those with hyperthyroidism from Graves' disease, toxic nodular goiter, or toxic adenoma.

The scientists found that the androsterone fraction was sharply increased in euthyroid patients when they were given thyroid hormone, and was decreased toward normal in patients whose hyperthyroidism was corrected by therapy.

The cholesterol-lowering effects of androsterone might seem paradoxical, since the androgens from which it is derived are normally associated with high serum lipid levels. However, the scientists point out, hormone metabolites are not necessarily inactive end-products of a spent hormone. They may have their own important physiological functions that are not related in any way to those of the substances from which they are derived.

These findings are the basis for an hypothesis, advanced by the scientists, that the recognizable effects of thyroid excess or deficiency are mediated by the availability of androsterone in the body. They also suggest that androsterone deficiency may be one of the mechanisms involved in the development of hypercholesterolemia and, perhaps, of atherosclerosis

New Technique Provides More Sensitive Measure of Plasma Clot Lysis

Using a sensitive, direct assay technique that closely approximates *in vivo* conditions, NHI grantees have measured the degree of clot-dissolving activity in human plasma They report in the *Journal of Clinical Investigation* (*39*: 426–434) that such activity depends primarily upon an activator whose concentration in plasma varies widely in response to stress, certain drugs, and disease.

For these studies Drs W D Sawyer, A P Fletcher, Norma Alkjaersig, and Sol Sherry, of the Washington University School of Medicine, St Louis, prepared clots from aged human plasma enriched with plasminogen and containing traces of bovine fibrinogen tagged with radioactive iodine The methods used produced I^{131} labeled clots that were largely free of entrapped human plasma

The clots were then incubated for two hours at body temperature in samples of unaltered plasma from healthy adults who had been subjected to stress or pretreated with certain drugs, and from patients with various chronic or infectious diseases. The degree of clot lysis was determined by measuring the amount of radioactivity released into each plasma sample as the clot dissolved

Thrombolysis is a complex process involving many factors whose exact roles have not yet been clarified The major components appear to be plasmin, the enzyme that actually dissolves the clot, plasminogen, its inactive precursor; and plasminogen activator, which converts plasminogen to plasmin. The circulating blood is thought to contain inhibitors for both active principles so that, under normal circumstances, they are present in the circulation in their "neutral" forms

A blood clot also contains plasminogen, which was trapped in the clot during its formation In fibrinolysis, it is thought, plasminogen activator accumulates on the fibrin surface of the clot and proceeds to convert the trapped plasminogen to plasmin. The plasmin dissolves the clot from within by digesting its fibrin.

Further knowledge of this property of plasma and better means of measuring it have been made essential by recent developments in the field of fibrinolytic drugs However, techniques heretofore available were indirect, were not sufficiently sensitive, or disturbed the complex balance between individual plasma components. The technique used in this study appears to avoid these limitations, and, with it the scientists have been able to provide experimental confirmation of concepts that formerly were largely inferential.

They found that plasma clot-lysing activity was sharply increased by sustained vigorous exercise and was increased by emotional stress or injections of nicotinic acid (a B-vitamin) or a fever-producing drug. It was also generally elevated in patients with acute infections, liver disease, or leukemia

Conversely, clot-lysing activity was commonly observed to be below normal in patients with cancer other than leukemia, congestive heart failure, or atherosclerosis This subnormal activity may be causally related to the thrombo-embolic complications that frequently attend these diseases

Plasma clot-lysing activity was found to be due primarily to the plasminogen activator it contained, and upon the plasminogen in the clot rather than that in the plasma. Clot lysis was greatly reduced when clots containing little plasminogen were incubated in the plasma samples, and was practically nil when plasminogen-free clots were used Clot lysis was also reduced by the addition of E-aminocaproic acid, which in low concentrations effectively inhibits plasminogen activator, but not plasmin

Clot-Dissolving Enzyme Isolated From Thrombin By NHI Grantees

Chemical "dissection" of thrombin has yielded a new clot-dissolving, or fibrinolytic enzyme identified as thrombin-E, report Drs. Walter H Seegers, Ricardo H. Landaburu, and J. Frederic Johnson at Wayne State University in Detroit The scientists observed powerful fibrinolytic effects of the new enzyme following its intravenous infusion in dogs.

Their findings published in *Science* (*131* 726) stem from an earlier observation that thrombin, long known for its important physiological role in catalyzing the formation of blood clots, has both the power to form and subsequently dissolve fibrin clots under certain laboratory conditions

In their currently reported studies, the investigators attribute the lytic activity of thrombin to a distinctly different enzyme which they named esterase thrombin, or thrombin-E, to distinguish it from thrombin-C, the parent compound.

They produced thrombin-E in pure form by acetylating thrombin-C with acetic anhydride. This destroyed the clotting power of thrombin-C and almost doubled the activity of thrombin-E, which was then stabilized by drying.

Dog experiments demonstrated the ability of intravenously infused thrombin-E to dissolve blood clots. In a typical experiment, 15 mg of dried thrombin-E dissolved in 100 ml of physiological saline solution, was slowly injected over a period of 1 hour and blood samples repeatedly withdrawn. Dr Seegers and co-workers report that at the end of the infusion period, blood samples clotted but subsequently lysed within an hour. "Occasionally the lytic phenomenon was so pronounced that the blood would not clot spontaneously upon withdrawal or even after thrombin was added," they stated The dogs tolerated the procedure very well; the few physiological effects observed, such as lowered platelet and white cell counts, increased blood sugar levels, and lytic phenomena, disappeared within 24 hours.

The investigators speculate that thrombin-E may function by activating physiological processes concerned with fibrinolysis and thus the maintenance of blood fluidity. "This opens possibilities for the useful application of thrombin-E," they said.

Arachidonic Acid Possible Factor in Resistance to Atherosclerosis

Studies by NHI grantees suggest that arachidonic acid—a highly unsaturated fatty acid found in certain fish oils, dairy products, and fowl—may be a significant factor in the relative immunity to athero-

sclerosis enjoyed by animals of certain species In these studies the cholesterol fractions of serum from man and from seven other species were analyzed and compared. The arachidonic acid content of serum cholesterol esters (which comprise about 70% of the total blood cholesterol) was found to be highest in those species most resistant to atherosclerosis, lowest in the most susceptible species.

The largest amounts of arachidonic acid were found in the cholesterol ester fractions of the rat (50%) and the dog (17%), both highly resistant species Much smaller amounts (1.1–5 8%) were found in the cholesterol esters of the goose, chicken, rabbit, guinea pig, and pig. Severe atherosclerosis can be produced in these species by feeding them high-cholesterol diets for only a short period, even though they seldom appear to develop the disease spontaneously.

Man (7.5%) is susceptible to atherosclerosis, but usually develops severe lesions only after many years on a diet high in fat and cholesterol (although much lower in cholesterol than the atherogenic diets fed laboratory animals).

However, the scientists point out, these findings, though in accord with an hypothesis that arachidonic acid deficiency is an important causal factor in atherosclerosis, have not proved that arachidonic acid is the protective factor nor that more of it in the diet of humans will make them less susceptible

But the possibility remains that the arachidonic acid content of human lipids may be increased by diets higher in this fatty acid or the elements from which it is synthesized (linoleic acid plus the co-factor vitamin B_6) It would then remain to be seen whether such an increase would confer upon man greater immunity to atherosclerosis

These studies were done by Dr Leon Swell, of the Veterans' Administration Center, Martinsburg, West Virginia; and Drs. Henry Field, Jr, and C. R Treadwell, of George Washington University. Their findings are reported in *Proceedings of the Society for Experimental Biology and Medicine* (*104* 325–328).

Find Serum Cholesterol Not Related to Obesity in Healthy Young Men

No significant correlation was found between serum cholesterol level and relative obesity in 159 male medical students at Johns Hopkins University, report Drs Caroline Bedell Thomas, Johns Hopkins University School of Medicine, Baltimore, and Stanley M Garn, Fels Research Institute, Yellow Springs, Ohio. Findings of their NHI grant-aided study appear in the journal *Science* (*131*: 42)

Obesity measured indirectly was expressed in the study as actual weight or weight in relation to skeletal chest-frame diameter as seen in chest X-ray films

Measurements of the fat-plus-skin thickness at the level of the tenth rib were also used by the investigators as a more direct method of determining obesity. The fatty layers were seen as shadows on the X-ray films and ranged in thickness from 2 to 15 mm., averaging 7.4 mm. thick

Serum cholesterol values varied in the students from 140 to 386 mg. per 100 ml. of serum and averaged 225 mg./100 ml.

The study revealed an insignificant correlation ("not significantly different from zero at the 5 percent level of confidence") between serum cholesterol levels and direct and indirect obesity measurements

"The lack of relationship between the amount of body fat and the serum cholesterol level demonstrated in the present study supports the view that the nutritional status of healthy young men such as medical students is considerably less important in determining the cholesterol level than other biologic factors," conclude the investigators.

Several of these "biologic factors," which include age, sex, race, heredity, endocrine patterns, habits of smoking and exercise, and emotional stress, have been previously reported by Dr. Thomas and co-workers to bear a positive relationship to high serum cholesterol levels among the Johns Hopkins medical students.

Find Mucopolysaccharides Not Causally Related to Atherosclerotic Lesions

Because mucopolysaccharides are often found in increased amounts at the site of atherosclerotic lesions, they have been suspected of being a causative factor in atheroma development. However, studies of the aortas of cholesterol-fed rabbits suggest that the increased amounts of mucopolysaccharides usually found in arterial tissue at the site of atherosclerotic lesions are secondary to fat deposition rather than a cause of it. The studies, supported in part by an NHI grant, were carried out by Drs A. J. Bollet, Wayne State University College of Medicine, Detroit; Chun Wang, Detroit Receiving Hospital, and David Adlersberg, Mount Sinai Hospital, New York Their findings are reported in *Circulation Research* (8 88-92)

Mucopolysaccharides are found in the connective tissues which bind together and support the cellular components of blood vessels and many other organs. The supportive function is accomplished by the protein collagen, whose insoluble fibers are embedded in a soluble matrix called the ground substance Mucopolysaccharides are important constituents of the ground substance.

In recent years studies on the possible role of the arterial ground substance in atherosclerosis have shown that the development of atherosclerotic lesions appears to be accompanied by increased concentrations of mucopolysaccharides in the affected tissues Animal studies

also showed that cortisone (which retards mucopolysaccharide metabolism) slowed the development of atherosclerotic lesions while the enzyme hyaluronidase (which breaks down the mucopolysaccharide hyaluronic acid) counteracted the effects of cortisone. These findings suggested that changes in mucopolysaccharides are important in atheroma formation and might even be a causative factor.

To investigate this possibility, the scientists induced atherosclerosis in rabbits by feeding them cholesterol for 1-7 months. Animals were sacrificed at two-week intervals after the first month and their aortas analyzed both for lipids and for mucopolysaccharides Early atherosclerotic changes were found in the aortas of animals after 1-2 months of cholesterol feeding, and moderate to severe lesions in animals fed cholesterol for three months or more. However, chemical analysis of the mucopolysaccharide content of these vessels showed no significant increases except in the more advanced lesions produced by six months or more of cholesterol feeding.

The findings suggest that alterations in mucopolysaccharides are indeed related to lipid changes in the development of atherosclerotic lesions, but are secondary to lipid deposition, not a cause of it

NHI Findings Support Filtration Theory of Atherosclerosis

Evidence supporting the filtration theory of atherosclerosis has been obtained from studies on experimental atherosclerosis conducted by Dr. Leroy Duncan, Mrs Katherin Buck, and Mr. Almorris Lynch, of the NHI Laboratory of General Medicine and Experimental Therapeutics

The filtration theory assigns the chief role in the development of atherosclerosis to lipoproteins, fat-protein complexes which serve as the major vehicles of lipid transport in the body. According to this theory, intact lipoproteins carrying cholesterol pass from the plasma into the inner layer of the arterial wall Here, because of the structure of the wall, they are trapped and give rise to atheromatous lesions

The studies on experimental atherosclerosis were based on information gained in previous studies on the movement of plasma albumin into arterial walls. These studies had disclosed that albumin enters the aorta with a gradient of rates, entering most rapidly in the upper aorta and progressively more slowly down its length The investigators reasoned that plasma albumin and plasma lipoprotein might enter aortic wall by the same mechanism and thus show the same gradient of rates When combined with the filtration theory, this reasoning led to the prediction that there should also be a gradient in the deposition of cholesterol along the length of the aorta early in the development of experimental atherosclerosis

Subsequent determinations of the cholesterol concentrations along

the length of aortas in dogs confirmed the prediction Early in experimental atherosclerosis there is a striking gradient of cholesterol deposition, and this gradient is very similar to the gradient of rates with which albumin enters the aortic wall. The experimental demonstration of this consequence of the filtration theory offers strong support for that theory.

The scientists found that the gradient disappears later in the develpment of experimental atherosclerosis, and that the cholesterol concentrations eventually become higher in the lower portion of the aorta This sequence of events suggests that the most severe atheromatous lesions tend to localize eventually in the lower aorta because of the extremely slow removal of cholesterol from that segment.

These studies, reported in *Circulation Research* (8: 1023-1027), also emphasize that the interplay of the rates of deposition and removal of cholesterol from the arterial wall are of prime importance in the development of atherosclerosis. Studies on factors influencing these rates are in progress

BLOOD PRESSURE

New Hypotensive Drugs Minimize Side Effects by Selective Action

Syrosingopine and guanethidine, two new hypotensive drugs synthesized and marketed by CIBA, have been found to be effective in lowering blood pressure and also specific enough in their modes of action to minimize undesirable side effects. Scientists of the NHI Laboratory of Chemical Pharmacology have found that both drugs lower blood pressure by depleting the peripheral sympathetic nerve endings of norepinephrine, thereby blocking the transmission of nerve impulses that trigger blood vessel constriction

Drugs that lower blood pressure by peripheral sympathetic blockade are not new; some have been in use for years Most of these work by inhibiting the action of norepinephrine, an amine that is released from sympathetic nerve terminals to transmit their impulses to the muscles of the arterial wall Reserpine was the first drug found to lower blood pressure by depleting this amine at sympathetic terminals, but only slightly larger doses may also produce undesired sedation and parasympathetic effects by depleting brain amines, and diarrhea by releasing serotonin in the intestines A major problem in treating hypertensives with reserpine has been the maintenance of dosage schedules that would hold blood pressure down without causing these side effects

Syrosingopine, a compound made by "tailoring" the reserpine molecule slightly, resembles reserpine in its effects on blood pressure, but is

more selective in its site of action. In experiments on animals, Drs Barbara H. Orlans, Kenneth F. Finger, and Bernard B. Brodie compared the effects of reserpine and syrosingopine on peripheral norepinephrine and on brain amine levels Both drugs depleted peripheral norepinephrine in doses that did not affect brain amines; however, reserpine exhibited this selectivity only over a narrow dosage range, whereas syrosingopine did not affect brain amines over a wide range. This more selective peripheral action of syrosingopine is a highly desirable clinical feature that provides a greater margin of safety against undesirable side effects

Guanethidine, a synthetic drug structurally different from reserpine, is also far more specific in its action than is reserpine The drug depletes the peripheral sympathetic nerves of norepinephrine, but does not affect brain amine levels (due in part to its limited ability to penetrate the blood-brain barrier), therefore does not produce sedation It does not release serotonin from peripheral body tissues, thus does not cause diarrhea. The drug is also well suited to oral administration because it is stable in the gut and is more readily absorbed than are ganglionic blocking agents.

The work by Drs. Rosemary Cass, Ronald Kuntzman, and Bernard B Brodie did not disclose the precise mechanism by which guanethidine depletes peripheral norepinephrine, even though the results indicate that it does so by a mechanism different from that of reserpine. There are two possibilities, both under study the drug may impair storage of the amine or block its synthesis

The findings on the two drugs are reported, respectively, in the *Journal of Pharmacology and Experimental Therapeutics* (*128*: 131–139) and in *Proceedings of the Society for Experimental Biology and Medicine* (*103*. 871–872)

Neurological Disturbances in Catron-Treated Patients Due to Common Amino Acid

Tryptophan is an essential amino acid found in many dietary staples, and which, under normal circumstances, produces no untoward effects on the consumer However, when given to patients receiving monoamine oxidase (MAO) inhibitors, tryptophan is capable of producing pronounced neurological disturbances, some of them strikingly similar to those of alcoholic intoxication

These disturbances were observed by Drs John A. Oates and Albert Sjoerdsma, of the NHI Laboratory of General Medicine and Experimental Therapeutics, after giving tryptophan orally to seven hypertensive patients being treated with Catron, a potent MAO inhibitor. The doses of tryptophan producing these effects were only 1½–3 times the usual dietary intake of this amino acid This small difference raises the question of whether diets unusually high in this or other

amino acids might not modify the clinical effects and side effects of MAO inhibitors in the treatment of hypertension and especially in the treatment of certain depressed states.

In three of these patients, tryptophan produced irregularities of co-ordination, euphoria, release of inhibitions, slurred speech, and other effects strikingly similar to those of alcoholic intoxication These and other patients also showed signs of drowsiness, a sharp contrast to the psychic energizing effects usually produced by MAO inhibitors Four patients receiving the largest doses of tryptophan also exhibited exaggerated reflexes, confined chiefly to the lower extremities, and accompanied by spasms of the ankle.

The fact that tryptophan produced these effects only in patients receiving MAO inhibitors suggests that the effects might be due to one or more of the amines produced from this amino acid. The most likely suspects are tryptamine and serotonin.

Animal studies have shown that doses of tryptophan increase the levels of both of these amines in the brain, and that this increase is augmented by MAO inhibitors. These inhibit the destruction of tryptamine and serotonin by the enzyme monoamine oxidase at the same time that tryptophan is leading to stepped-up amine production. Increased levels of either or both amines are accompanied by neurological effects that vary according to the species of animal

In man the evidence is less direct, but tryptophan has been shown to increase urinary excretion of tryptamine and an end-product of serotonin metabolism 5-hydroxyindole acetic acid (5-HIAA). Thus it is reasonable to assume that levels of both amines in the nervous system are increased by tryptophan and MAO inhibitors, and that either or both amines may be responsible for the observed neurological effects

These findings have been accepted for publication in *Neurology*

Chronic Renal Hypertension Not Dependent on Pressor Substances From Kidney

Animal studies on the role of the kidney in hypertension have provided evidence that the chronic elevation of blood pressure that persists in renal hypertension is not dependent on increased blood levels of artery-constricting substances from the kidney. These studies, supported in part by an NHI grant, are reported in the *American Journal of Physiology* (*198.* 1148-1152) by Drs. Pedro Blaquier, David F Bohr, and Sibley W Hoobler, of the University of Michigan.

The kidney is known to be the villain in several forms of secondary hypertension and has also been suspected of playing a role in the development and/or maintenance of essential hypertension It has been demonstrated that the kidney releases renin as an acute response

to an inadequate renal blood supply; and that renin, in turn, reacts with a serum protein to form angiotensin, a potent artery constrictor. But whether this response is transient or is involved in the maintenance of chronic hypertension has been the subject of much conflicting evidence.

In the study of this problem, the scientists used a cross-circulation technique in which the femoral arteries and veins of rats with surgically produced renal hypertension were connected by plastic tubing to those of normotensive rats Preliminary work had shown that (1) cross circulation between pairs of normotensive rats produced no significant changes in blood pressure, and (2) elevated blood pressure produced in one of the pair by infusions of renin or angiotensin was followed shortly by elevated blood pressure in the other. Thus, if the hypertension in the surgically treated rat were due to circulating vasoconstrictors, cross circulation should also produce hypertension in the normotensive rat of each pair.

To rule out blood pressure aberrations that might be due to blood volume changes, normal and stable blood volumes were maintained by equalizing the flow rates between the animals of each pair by adjustable clamps on the plastic tubing. The animals were arranged on the two pans of a sensitive balance so that any volume variations would be promptly noted and corrected

In one series of experiments the scientists used seven rats whose renal arteries had previously been clamped for four hours. When the clamps were removed, hypertension was produced within minutes, presumably by renin from the ischemic kidney. Cross circulation, immediately initiated between these rats and seven normotensives, resulted in elevated blood pressure in all normotensive rats. However, cross circulation between normotensive rats and those with hypertension of more than one week's duration had no significant effect on the blood pressure of the normotensives.

Thus, it appears from these experiments that circulating artery constricting substances such as renin may play some role in the *initiation* of renal hypertension, but may not contribute to its maintenance

Diet-Induced Hypertension in Rats Similar to Human Essential Hypertension

Studies on rats made hypertensive by diets deficient in potassium or choline during early life suggest that this dietary technique may yield laboratory animals more suitable for research on the role of the kidney in essential hypertension than do commonly used methods involving renal artery constriction or removal of one kidney. Renal function studies on rats showed that the diet-induced hypertension in

these animals, like essential hypertension in humans, was not the result of an inadequate renal blood supply nor was it associated with severe excretory insufficiency.

In these experiments, Drs Fred N. White, M. P. Sambhi, and Arthur Grollman, of the University of Texas Southwestern Medical Center, studied renal function in 31 rats: 10 normal controls and 21 with diet-induced chronic hypertension. The NHI grantees compared data from the two groups to determine the effects of the experimental hypertension on rates of renal blood flow, plasma filtration by the kidney glomeruli, and excretion of urea.

Their findings, reported in the *American Journal of Physiology* (*198* 221-222), revealed significant changes from normal only in the glomerular filtration rates, which were somewhat reduced in the hypertensive rats The findings suggest certain striking similarities, with respect to renal function, between diet-induced experimental hypertension and the essential hypertension that develops spontaneously in humans. Neither is the primary result of an inadequate renal blood supply and neither is accompanied by severe impairment of excretory capacity

These similarities suggest that the dietary technique might yield laboratory animals especially suitable for research on kidney function and hemodynamics in hypertension, especially on more subtle renal changes that might be masked by the severe lesions and renal ischemia usually resulting from drastic surgical procedures Furthermore the dietary procedure is simpler and less subject to failure than are most surgical techniques. The potassium or choline deficient diets need to be fed to the animals for only a short period (usually 1-5 weeks) early in their lives to produce hypertension, after which the disease is self-maintaining and progressive.

Previous studies by Drs Grollman and White, who developed the technique, indicate that the morphological changes induced in the heart, blood vessels, and kidneys by diet-induced hypertension are also similar to those of essential hypertension in humans. Thus the technique may aid in the accumulation of experimental data that might establish relationships between experimental hypertension in animals and essential hypertension in man Such relationships might help to clarify many of the puzzling features of this enigmatic disease

Studies Demonstrate Value of Renal Revascularization in Selected Hypertensives

Hypertension caused by narrowing of one or both main renal arteries has been relieved in 33 of 40 patients by restoring blood flow to affected kidneys, report NHI grant-aided investigators in Houston, Texas.

In renovascular hypertension caused by occlusive disease of one or

both main renal arteries, the affected kidney(s) releases a substance (renin) which acts in the bloodstream to form the hypertensive agent, angiotensin Patients with this kind of hypertension involving only one kidney have been treated by removal of that kidney (nephrectomy). However, because nephrectomy relieved hypertension in only 20 percent of these patients and could not be applied to bilaterally-affected patients, it was abandoned more than a decade ago In recent years, surgical treatment of renovascular hypertension has been revolutionized by the development of procedures for conserving renal function by removing or bypassing arterial obstructions

The value of this modern surgical approach to renovascular hypertension was recently demonstrated in studies conducted by Drs G. C Morris, Jr, M. E. DeBakey, J W Overstreet, and Russell Scott, Jr., of the Baylor University College of Medicine in Houston, Texas They used renal arteriography for the definitive diagnosis of renovascular lesions in 40 hypertensive patients. The lesions, which affected both kidneys in 40 percent of the patients, were then corrected in one of two ways in most cases the obstructions were bypassed by synthetic grafts of Dacron, narrowed arteries were also widened by cutting them longitudinally and inserting a Dacron patch-graft into the incision Results of surgery, evaluated by physical examination, renal arteriography, and tests of renal function, are reported in the *American Surgeon* (26 745-749)

Dr Morris and co-workers found that 33 (82 percent) of these patients became normotensive and have remained so (observation periods at the time of report were up to 3 years' duration) Of the remaining 7 patients, 4 showed some improvement in their hypertension and 1 patient died 5 days postoperatively as the result of a coronary occlusion They also report " renal function following revascularization for unilateral involvement is always superior on the side of renal artery narrowing," a finding which emphasizes the value of conserving renal function in unilaterally as well as bilaterally affected patients

SURGERY

Cardiac Shunts Detected by Heart Injections of Krypton-85

Studies by NHI scientists on 48 patients have shown that radioactive krypton, injected directly into the heart via a cardiac catheter, provides a simple, rapid, and accurate means of detecting and localizing cardiac shunts The small quantities of the isotope needed for the procedure and its short biological half-life make the method safe both for the patient and for laboratory personnel.

Shunts result from holes in the partition separating the right heart from the left heart. The right heart receives oxygen-poor blood from the body and pumps it to the lungs; the left heart receives the freshly oxygenated blood from the lungs and pumps it to the body. By allowing the mixing of oxygen-poor blood with oxygenated blood in the heart, shunts can seriously impair the efficiency of the heart in delivering oxygen to the tissues.

To detect left-to-right shunts, a catheter is positioned in the left heart and 3-5 ml. of saline containing dissolved krypton-85 injected. If no shunts exist, the gas will be pumped throughout the system and largely dissipated before making a much-delayed appearance in the lungs via the right heart. But a left-to-right shunt will allow some of the gas to cross to the right heart, where it is pumped directly to the lungs Thus, shortly after injection the gas will appear in high concentration in the air exhaled by the patient. The radiation of the expired air is monitored by a count-rate meter and recorded continuously on a direct-writing oscillograph

To detect right-to-left shunts. the krypton-85 is injected into the right heart and arterial blood samples drawn at a constant rate for the next 15 seconds In the absence of a shunt, all of the gas is pumped to the lungs where about 95% of it is eliminated in the expired air before entering the arterial circulation. Thus, high concentrations of the isotope in the arterial samples mean that the gas bypassed the lungs by crossing directly to the left heart

Repeated injections of krypton-85 with the catheter tip stationed at different points makes it possible to localize shunts with a considerable degree of accuracy The method also permits estimation of the magnitude of right-to-left shunts by relatively simple calculations

In every patient in which this technique indicated the presence or absence of a shunt, its accuracy was subsequently verified by findings at operation or autopsy, or by the use of other established techniques for diagnosing shunts These included selective angiography, dye dilution curves, and krypton-85 or nitrous oxide inhalation tests. The method proved to be convenient, simple to apply during heart catheterization, and sensitive enough to permit the detection and localization of even very small shunts

These clinical studies were conducted by Drs R T L Long, Eugene Braunwald, and A. G Morrow, of the NHI Surgery Branch Their findings are reported in *Circulation* (*8* 1126-1133)

Superiority of Butanol Cardioplegia Revealed by Animal Studies

The frequently observed depression of heart action in patients following elective cardiac arrest has stimulated the quest for better

methods of stilling the heart Chemical methods (aortic injections of potassium citrate or acetylcholine) have been largely abandoned in favor of the anoxic method which involves clamping the aorta to restrict bloodflow to the heart. Now evidence that butanol-induced cardioplegia has certain advantages over the anoxic method is reported in the *Transactions of the American Society for Artificial Internal Organs* (6: 323-337) by Drs H. H McGuire, Jr., L. H. Bosher, Jr, and R W. Ramsey, of the Medical College of Virginia, Richmond. Their studies were supported by a National Heart Institute grant.

The scientists divided 21 dogs into four groups according to type and duration of cardioplegia butanol arrest of 15 or 30 minutes duration, and 15 or 30 minutes anoxic arrest Control ventricular function data was obtained for each group prior to arrest for comparison with similar data obtained 15 and 30 minutes after cessation of arrest.

Comparison of the post-arrest ventricular function curves showed that recovery following 15-minute arrest was much more rapid in the butanol-treated animals than in the anoxic arrest group At 15 minutes post arrest, for example, ventricular function had returned to 85% of normal in the butanol-arrested animals, but to only 49% of normal in the others Recovery after 30-minute butanol arrest was less satisfactory, but was still much more rapid than that after 30-minute anoxic arrest

Although the protection conferred by butanol appears to diminish if cardioplegia is maintained for longer than 15 minutes, the scientists feel that this protection might be extended for longer periods by the use of hypothermia or auxiliary metabolic or cardioplegic agents

New Catheter Design Permits Selective Visualization of Coronary Arteries

A new coronary arteriography procedure, involving the use of a special loop-end catheter, is reported in *The New England Journal of Medicine* (262 325-333) by NHI grant-aided scientists in Boston Their studies in dogs and humans show that the technique permits safe, selective X-ray visualization of the coronaries with smaller amounts of radiopaque dye than is usually required by other procedures Further application of this technique may improve X-ray diagnosis of coronary artery disease

The Boston scientists are Drs Sven Bellman, H A. Frank, P B Lambert, David Littmann, J H Hall, and J A. Williams of Beth Israel Hospital, West Roxbury Veterans Administration Hospital, and Harvard Medical School.

In coronary arteriography, a radiopaque substance is injected through a catheter into the base of the aorta where the coronary

arteries branch out into the heart. As contrast substance flows into the coronaries, rapid-sequence X-ray pictures are taken to reveal the extent and location of atherosclerotic deposits. Success of the procedure depends upon an adequate flow of contrast medium into the coronaries, and has been accomplished in the past by either injecting large quantities of medium or by diverting more contrast material into the coronaries through aortic occlusion or temporary heart arrest. While the procedure is usually safe and reliable, adverse reactions have been observed in some patients receiving large dye injections.

The new technique employs a different principle of injection whereby dye is injected through holes in the catheter loop and directed toward the coronary openings, thus eliminating the necessity for large amounts of dye. The loop-end catheter was developed by the scientists after their dog studies had shown that ordinary, straight-tipped catheters recoil from the coronary openings as dye is injected

The catheter loop is held straight by a wire guide while being inserted into the aorta and regains its circular shape upon withdrawal of the guide. The synchronous injection of dye and rapid-sequence exposure of X-ray plates may be repeated for a second set of films.

The Boston workers report that coronary arteriography with the loop-end catheter was well tolerated by both dogs and humans, and that good coronary visualization was regularly obtained They expressed the belief that this method is inherently safer than other methods currently available, and concluded, "This approach to the problems of angiographic diagnosis of coronary artery disease shows considerable promise"

The loop-end catheter method is one of several recent developments in coronary arteriography and illustrates progress being made in this field

Technique Increases Safety of Surgery To Unblock Small Arteries

A new technique that permits surgical removal of atherosclerotic deposits, clots, or other lesions obstructing blood flow in small arteries has been developed by Drs E S Crawford, A C Beall, P R Ellis, Jr., and M E DeBakey of the Baylor University College of Medicine The new method overcomes the problem formerly posed by the constriction of the inner diameter of the artery at the site of the incision during the healing process This is done by inserting a knitted Dacron patch at the incision site (following removal of the arterial obstruction), then suturing the wound edges to this patch rather than to one another

After being tested on laboratory animals, the technique was used in 93 patients suffering from lesions impeding arterial blood flow At the

site of arterial blockade a longitudinal incision was made through the artery wall, cutting through all of the diseased portion plus a short undiseased arterial segment at either end. A piece of plastic tubing was inserted to detour blood around the site while the obstruction was removed The Dacron patch was then inserted and the wound edges sutured to its circumference, the plastic tubing "detour" being withdrawn just before the final suture was drawn taut.

None of these patients developed recurrent arterial obstruction during their convalescence, and only two had recurrences during the next 30 months This is in sharp contrast to the results of animal studies in which incisions, made in arteries of comparable diameter, were repaired by suturing the wound edges together Every artery repaired in this way subsequently became constricted

Endarterectomy, the direct surgical removal of lesions obstructing blood flow, has been used successfully to restore normal circulation in such large arteries as the aorta and iliac Theoretically, the same technique should be even better suited for obstructed small arteries, such as the coronary, renal, or vertebral arteries, since lesions tend to be confined to short segments of such vessels. However, its application had previously been sharply limited by the constriction that often developed in such vessels after surgical repair—a factor of great importance in small vessels because of the attendant danger of clot formation

By overcoming this problem, the new technique greatly extends the use of surgery for the correction or improvement of arterial insufficiency resulting from atherosclerosis or other cardiovascular diseases. It may also make possible the safe and effective use of surgery in the treatment of coronary heart disease, heretofore attended by high risk and low probability of success

This work, which was supported in part by an NHI grant, is reported in *Surgical Forum* (*10* 671-675)

Combat Heart Arrest by Massage Through Unopened Chest

A new method of closed-chest cardiac massage which may be used by "anyone, anywhere" to restore blood flow in patients with cardiac arrest, is described in *The Journal of the American Medical Association* (*173* 1064-1067) by Drs W. B Kouwenhoven, J R Jude, and Mr G G. Knickerbocker of the Johns Hopkins University School of Medicine at Baltimore The method, which they developed through extensive experimentation on more than 100 dogs, has been used with notable success on patients with cardiac arrest

The new technique is easily applied and, unlike direct cardiac massage, it eliminates the necessity for surgical exposure of the heart. To

effect massage, the operator places both hands, one above the other, with the heel of the lower hand resting on the lower third of the patient's sternum and presses vertically downward about 60 times per minute. With each pressure stroke the sternum is moved 3 or 4 centimeters toward the spine, thus compressing the heart and forcing out blood Relaxation at the end of the stroke allows the heart to fill again Artificial respiration should be given simultaneously.

"When cardiac arrest occurs," the scientists point out, "the circulation must be restored promptly, otherwise anoxia will result in irreversible damage." They found that closed-chest cardiac massage, combined with artificial respiration, maintains a flow of oxygenated blood to the heart and central nervous system until normal heart action returns spontaneously or, in cases of ventricular fibrillation, until a closed-chest defibrillator can be brought to the scene of the emergency. According to the investigators' experience, hearts in fibrillation for longer than 3 minutes usually do not respond to defibrillation procedures. Closed-chest cardiac massage, however, greatly extended this time limit: "Adequate circulation for periods as long as 30 minutes was easily maintained with the dog in ventricular fibrillation. A closed-chest defibrillating shock would result in the immediate return of normal sinus rhythm in such animals," they explain

In the 10 months preceding their report, Dr. Kouwenhoven and co-workers applied closed-chest cardiac massage to 20 patients who were from 2 months to 80 years of age. In addition to cardiac massage, which varied from less than 1 minute to 65 minutes in duration, 3 patients required defibrillation and 13 required artificial respiration The scientists report that all 20 patients were successfully resuscitated and, at the time of this report, 14 patients were alive without central nervous sysem damage; an over-all permanent survival rate of 70 percent

Although their data was obtained from hospitalized patients, Dr Kouwenhoven and co-workers conclude, "The real value of the method lies in the fact that it can be used wherever the emergency arises, whether that is in or out of the hospital."

Portable Pacemaker Aids Patients With Complete Heart Block

Electrical stimulation of the heart with a transistorized portable pacemaker is reported to be an effective method of treating complete heart block in patients A description of the apparatus and its use in 66 patients is reported in *The Journal of the American Medical Association* (*172* 2006–2010) by Drs C W Lillehei, V L Gott, P C Hodges, Jr, D M Long, and Mr E E Bakken of the University of Minnesota Medical School at Minneapolis

In complete heart block, disease or operative injury of the heart's conduction tissue blocks nerve impulse conduction from the sinoatrial node (the heart's "pacemaker" located in the right atrium) to the ventricles. The ventricles thus lose their normal rhythm and frequently cease beating entirely.

The artificial pacemaker developed by the Minnesota workers is designed to maintain the heartbeat for days, weeks or even months—until diseased or injured tissue regenerates and the heart's own pacemaker can resume maintenance of normal heart rhythm The device consists of a transistorized transformer powered by a self-contained long-life battery, and is connected to the heart by one or two thin wire electrodes inserted into the heart muscle.

Electrode(s) may be inserted either through the open chest or, in nonsurgical patients, by piercing the chest wall with a hollow needle, through which an electrode is passed and the needle subsequently withdrawn. The small pacemaker (slightly larger than a package of cigarettes) is completely portable and is worn strapped to the patient's chest

The NHI grant-aided scientists report that artificial pacemaker stimulation has proved to be the most effective method of managing complete heart block in surgical patients, and that $\frac{2}{3}$ to $\frac{3}{4}$ of these patients required less than 2 or 3 weeks of continuous stimulation. Most of the remaining surgical patients were "weaned" from the pacemaker by progressively lowering the rate of stimulation and administering isoproterenol hydrochloride, a drug which increases cardiac rhythmicity and rate

Most of the patients treated in this series had developed heart block subsequent to cardiac surgery However, the pacemaker has also been used successfully in the treatment of Stokes-Adams syndrome—heart block due to atherosclerosis, infection, or certain drugs—restoring many of its victims to useful activity The investigators point out that, not only has the threat of sudden death in these patients been removed, but that their physical and emotional rehabilitation has been dramatic At the time of their report, one patient with Stokes-Adams syndrome had been maintained under continuous stimulation for 15 months

New Aortic Valve Prostheses Reported by NHI Grantees

University of Minnesota surgeons David L Long, Jr., Laurence P Sterns, Robert H De Reimer, Herbert E Warden, and C. Walton Lillehei, described in *Surgical Forum* (10 660–665) two different types of plastic valves which they developed for the replacement of irreparably damaged aortic valves They report that one type of valve has been used with success in patients

Both valves—a flap valve and a bicuspid valve—are constructed of silastic (a rubbery silicone plastic) bonded to a polyvinyl sponge (Ivalon) base. They are inserted through a slit in the aorta and secured by sutures at the site of the natural aortic valve.

Both valves have been tried in dogs They were also tested in human cadaver hearts which were made to "beat" in a mechanical pulse duplicating apparatus.

Flap valves were successfully inserted by the Minnesota surgeons in two patients, with subsequent restoration of valvular function in each case. The investigators report that the first patient was well and active at the time of their report, eleven months after surgery, and that the second patient was well and active following the operation but died two months later of an unrelated cause. Autopsy revealed a properly positioned, functional valve with no abnormal blood clots present.

Function and durability tests of the bicuspid valve in a pulse duplicating machine have been very encouraging but complications that occurred upon installation of the valve in dogs delayed clinical trials Difficulties encountered included leakage of blood between the valve and aortic wall in some animals before a suitable method was devised for securing the valve. Complete heart block also developed in some animals as a result of damage to heart impulse-conduction tissue by sutures or internal bleeding The investigators believe that, in the human heart, the larger size of the structures may make it possible to avoid this complication.

Results of the Minnesota studies suggest, conclude the investigators, "that substantial progress has been achieved in the resolution of many of the very significant problems concerned in the use of artificial cardiac valves Moreover, this same progress, culminating in successful clinical application, has served to point the way for numerous further improvements in choice of materials, design, and surgical technique."

Artificial Heart Prototypes Designed and Tested by NHI Grantees

The design and preliminary trials of two prosthetic hearts are reported in the *American Heart Journal* (59 723-736) by Drs C S Houston, Tetsuzo Akutsu, and W J Kolff, at the Cleveland Clinic Foundation and the Frank E. Bunts Educational Institute in Cleveland Studies of the mechanical hearts in dogs and in a mock circulatory system indicate that progress is being made toward replacing irreparably damaged human hearts with mechanical pumps implanted within the chest These pumps, however, are not sufficiently developed to warrant their installation in humans

Each of the new prostheses—a pendulum-type pump and a roller-type pump—contains an electric motor and two elastic pumping cham-

bers, or ventricles, of polyurethane plastic. Polyurethane valves and connecting tubes maintain unidirectional blood flow through the hearts, and plastic coated wires supply current from an externally located battery pack or wall socket to power the hearts.

In the pendulum pump, a motor suspended on pivots (the "pendulum") swings back and forth within a rigid outer housing, alternately compressing each ventricle against the housing. To-and-fro excursions of the motor-pendulum are caused by an eccentrically mounted, adjustable lever arm which connects the motor shaft with the outer housing

Tests of the pendulum pump in a mock circulatory system showed that as much as 1800 ml of blood per minute is ejected from each ventricle and that hemolysis of red blood cells by the pump is not excessive, report the Cleveland scientists.

Encouraged by these tests, they installed a pendulum pump at the anatomical site of the natural heart in a 30-kilogram dog, which was maintained by a heart-lung machine during removal of its own heart. The investigators report that the dog's circulation was sustained for 5 hours by the pump, but that several infusions of norepinephrine were required to maintain blood pressure The pump had been adjusted to deliver 2000 ml per minute which, they explain, was inadequate for a dog of this size The pendulum pump, however, "seemed promising enough to serve as a model for a more durable pump, which is under construction," they report

The second type of pump designed by the Cleveland group employs a small, motor-driven roller to compress the ventricles against a rigid outer housing Polyurethane foam inserted between the ventricles and housing, provides a yielding surface which minimizes hemolysis

In mock circulation tests of this pump, ventricular outputs increased (in the left ventricle, from 200 to 2,200 ml per minute) as atrial pressures were increased Thus, the ouput on both sides are self-regulating, if one side pumps more blood than the other, the increased atrial pressure on the other side leads to an increased output by that side

In a single animal experiment, the roller pump sustained a dog for 2 hours Although the pump was not sufficiently streamlined to allow closure of the dog's chest, there was evidence of spontaneous breathing and of excellent blood oxygenation, pressure tracings indicated good functioning of the pump

Report First Successful Kidney Transplant Between Nonidentical Twins

Transplantation of a healthy kidney from a 24-year-old man to his fraternal twin brother has saved the life of the recipient, whose own incurably diseased kidneys were subsequently removed This operation,

the first successful renal transplant between nonidentical twins, was reported in the *New England Journal of Medicine* (262. 1251-1260) by Drs J. P. Merrill, J E. Murray, J. H. Harrison, E. A. Friedman, J. B. Dealy, Jr., and G. J. Dammin, of Peter Bent Brigham Hospital and Harvard Medical School in Boston. Their success follows 5 years of pioneering experience in transplanting kidneys between identical twins.

In the currently reported case, the brothers were believed to be fraternal rather than identical twins because of differences in their physical appearance. Proof of this, and of the tissue incompatibility that exists between individuals of different genetic background, were demonstrated by slow rejection of skin grafted from the donor to his brother and by accelerated rejection of a second graft. With the failure of these grafts, the Boston scientists realized that transplanted kidney tissue would be subject to the same fate unless the patient's immune response were modified in some way

Knowing that the rejection of tissues foreign to the body is a function of the antibody-forming system (blood-forming and lymphatic tissues) and that this system is depressed by X-rays, the scientists believed that immunological tolerance could be produced by the use of sublethal, whole-body irradiation that would temporarily impair, but not destroy the antibody-forming system. They postulated: "A kidney transplanted immediately after the administration of such irradiation would then constitute an antigen to which antibody production was impaired, if not destroyed." The slow recovery of antibody-forming capacity in the presence of foreign kidney tissue might correspond to the development of immunological tolerance in animals that have been injected with foreign cells during embryonic or neonatal life before the antibody-forming system is fully matured and capable of "recognizing" foreign tissue Tolerance thus acquired persists in adult life and enables acceptance of subsequent tissue grafts from the same donor.

On this theoretical basis, irradiation totalling 450 r was administered to the patient prior to kidney transplantation The kidney functioned immediately after restoration of its blood supply (it had been ischemic for a period of 52 minutes) and excreted 32 liters of urine during the first 36 hours. Eleven days later the patient's own diseased kidneys were removed, and within 2½ months he was completely well The patient continued to feel well despite the onset of graft rejection which was detected by urinary abnormalities and renal biopsy findings 10 months after operation. This beginning rejection was aborted by large doses of cortisone and small doses of irradiation, the patient quickly regained normal renal function, report the scientists

They conclude: "Partial tolerance for a renal homograft has been produced, and this tolerance is consistent with normal renal function and clinical well-being"

Thermal Dilution Curves Used Clinically To Diagnose Cardiac Shunts

Thermal dilution curves have been used successfully by scientists of the NHI Surgery Branch for the diagnostic evaluation of circulatory shunts in patients with congenital heart disease. The basic technique was not developed at NIH, but was modified here and new instrumentation developed for its application to clinical diagnosis. The technique is a substantial improvement over standard dye dilution methods because, while retaining the accuracy of these methods, it eliminates the necessity of withdrawing large blood samples and also the problems of skin discoloration and allergic reaction that occasionally follow multiple dye injections These studies are reported in the *American Journal of Cardiology* (6. 1065–1069) by Drs. Theodore Cooper, Eugene Braunwald, and Andrew H. Morrow, of the NHI Surgery Branch, and G C Riggle, of the Division of Research Services

Dye dilution curves are widely used to diagnose, localize, and estimate the magnitude of cardiac shunts, the abnormal flow of blood through holes in the partition that separates the two sides of the heart. In this technique dye is injected into the appropriate side of the heart and its concentration subsequently measured in blood withdrawn from a vessel located downstream from the injection site. As the blood is withdrawn at a constant rate into a special syringe, its dye concentration is continuously measured by a densitometer. If no shunt is present, the dye concentration plots as a curve with a sharp upslope followed by a smooth, gradual downslope However, a shunt causes a break in the curve or a second peak resulting from the appearance of dye detoured by the shunt Though accurate and safe, this method requires the withdrawal of large blood samples, and frequently necessitates blood transfusions, especially in young children Multiple determinations, frequently needed for precise diagnosis are also limited with this technique because of decreasing accuracy and increasing danger of allergic responses

The new technique uses cold saline as the indicator and measures its concentration as a function of blood temperature in a vessel downstream from the point of injection by means of a heat-sensing element called a thermistor Since the thermistor can be introduced directly into the vessel with a special needle or short catheter, no blood samples need be withdrawn The comparison by the NHI scientists of the new technique with standard dye dilution methods showed that the thermal dilution curves are strictly analogous to dye dilution curves and appear to be equally sensitive and accurate for the diagnostic evaluation of shunts Repeated determinations can be made with this technique without any decrease in accuracy and without any danger of toxicity, features which make possible the more precise diagnosis of shunts as well as immediate evaluation of surgical measures to correct them.

New Diuretic Is Effective Against Edema of Normal or Toxemic Pregnancy

Hydrochlorothiazide, a new diuretic drug derived from chlorothiazide, has been found highly effective against the edema of normal or toxemic pregnancy.

In NHI grant-supported studies at the University of California Medical Center, Dr. N. S Assali used hydrochlorothiazide, as Esidrex (CIBA) and HydroDiuril (Merck, Sharp & Dohme), to treat 33 patients with edema accompanying normal pregnancy and 25 edematous patients with toxemia of pregnancy. His findings, reported in *Clinical Pharmacology and Therapeutics* (*1:* 48–52), indicate that the diuretic action of the new drug closely resembles that of chlorothiazide, but appears to be effective at one-tenth the dose

Edema is the accumulation of fluid in the extracellular tissue spaces Its most common cause in normal pregnancy is too much salt in the diet or salt retention by the kidneys Edema in toxemia of pregnancy is also associated with salt retention, and may be abetted by the loss of protein in the urine that also characterizes this disorder. Although slight or moderate edema is more unsightly than clinically dangerous, severe edema can lead to fluid accumulation in the abdominal cavity (ascites) or in the chest, where it can seriously interfere with respiration.

Like chlorothiazide, hydrochlorothiazide reduced edema by increasing renal excretion of salt, potassium, and water, but 100–150 mg. seemed to produce effects comparable to those produced by 1000–1500 mg of chlorothiazide in a previous study The drug appears to increase salt and water excretion by inhibiting their reabsorption in the kidney tubules, since it produced no significant effects on renal plasma flow or filtration rates

The drug produced no observed adverse effects, did not noticeably alter serum electrolyte levels in most patients, and produced most marked diuresis in patients whose edema was most severe As edema diminished, the effects of the drug were reduced This is a very desirable feature of the drug because it provides a margin of safety to protect against the possibly serious consequences of electrolyte depletion.

Developments Extend Use of Gas Chromatography In Lipid Analysis

Important new contributions by NHI scientists have made possible the use of gas chromatography for the radioassay of fatty acids labeled

with radioactive carbon and for the analysis of steroids These developments should greatly facilitate research on lipids and lipid metabolism and the possible roles of these factors in atherosclerosis.

Originally developed in England in 1952, gas chromatography has helped solve many problems of lipid analysis formerly posed by analytical methods that were complex, slow, and insufficiently accurate for the assay of small samples. In gas chromatography, a small sample of the lipid mixture to be analyzed is injected into an analytical column, vaporized, and swept through the column on a stream of inert carrier gas (argon or helium)

In transit through the column the lipid vapors are brought into repeated contact with a non-volatile liquid, present as a thin coating either on the inner surface of the column or on small inert particles with which the column is packed All of the lipids are soluble in this liquid, but the extent varies with their structure and chemical properties This variation affords the means of separating the lipid components (the more soluble the lipid, the slower its transit and vice versa) and also for identifying each component as it emerges. As it emerges from the column the lipid vapor enters a special detector which senses its passage, measures its concentration, and records these data against time on a strip chart.

The system for the radioassay of carbon-14 labeled fatty acids by gas chromatography was worked out by Drs. Arthur Karmen and Harold R. Tritch, of the Laboratory of Technical Development The system, described in *Nature* (*186* 150-151), uses a short column containing anthracene crystals coated with silicone oil to trap the fatty-acid vapors emerging from the analytical column.

The radioactive vapor dissolves in this oil, thereby coming in close proximity to the anthracene crystals The radiation emitted causes small flashes (scintillations) in the crystals These scintillations are detected by phototubes and counted by an automatic scintillation counter

The system allows the collection of individual fatty acid esters in separate trapping columns or for continuous monitoring of a single column In the latter method, a device measures the rate at which scintillations are occurring and plots this against time on a strip chart As each new component enters the trapping column, this rate increases sharply From this data can be determined the time that each component entered the column and how much radioactivity it contained

The use of gas chromatography for the analysis of steroids was made possible by a special analytical column designed by Drs W J A VandenHeuvel, C C Sweeley, and E C Horning, of the NHI Laboratory of the Chemistry of Natural Products

Steroids, the subject of intense scientific study in recent years, are members of a large family of compounds which include cholesterol, many important adrenal hormones, several vitamins, and the male and

NEW KNOWLEDGE AND METHODS

New Diuretic Is Effective Against Edema of Normal or Toxemic Pregnancy

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Edema is the accumulation of fluid in the extracellular tissue spaces Its most common cause in normal pregnancy is too much salt in the diet or salt retention by the kidneys. Edema in toxemia of pregnancy is also associated with salt retention, and may be abetted by the loss of protein in the urine that also characterizes this disorder Although slight or moderate edema is more unsightly than clinically dangerous, severe edema can lead to fluid accumulation in the abdominal cavity (ascites) or in the chest, where it can seriously interfere with respiration

Like chlorothiazide, hydrochlorothiazide reduced edema by increasing renal excretion of salt, potassium, and water, but 100–150 mg seemed to produce effects comparable to those produced by 1000–1500 mg. of chlorothiazide in a previous study. The drug appears to increase salt and water excretion by inhibiting their reabsorption in the kidney tubules, since it produced no significant effects on renal plasma flow or filtration rates.

The drug produced no observed adverse effects, did not noticeably alter serum electrolyte levels in most patients, and produced most marked diuresis in patients whose edema was most severe As edema diminished, the effects of the drug were reduced This is a very desirable feature of the drug because it provides a margin of safety to protect against the possibly serious consequences of electrolyte depletion

Developments Extend Use of Gas Chromatography In Lipid Analysis

Important new contributions by NIH scientists have made possible the use of gas chromatography for the radioassay of fatty acids labeled

according to the physical properties of the gas, such as its molecular weight. With the equipment described, voltage is applied to a sound-transmitting crystal at one end of a tube containing the sample, and recorded by a receiving crystal at the other end. Phase differences induced by particular samples between the two voltages are sufficiently definitive to imply analytical usefulness for the principle

The actual value of the sound velocity principle as used in the equipment Mr. Noble described remains to be demonstrated in applied research

Drug Metabolism by Liver Unaltered in Patients With Cirrhosis

Preliminary studies at NHI and New York University suggest that normal rates of drug metabolism are not altered by cirrhosis, despite severe loss of functioning liver, wherein are located the special enzyme systems that inactivate drugs. Thus, the presumed sensitivity to certain drugs of patients with liver disease may require some other explanation, if these indications are borne out by more extended studies

Conversion of certain fat soluble foreign compounds, such as drugs, into inactive derivatives is an important function of the liver, without which the action of many drugs would persist for a dangerously long time The liver disposes of such compounds by converting them to derivatives which are less fat soluble, thus barring them from reabsorption into the body through the fat-like membrane lining the kidney tubules

Recently Dr Bernard B Brodie and Dr John Burns of NHI, and Dr Murray Weiner of New York University Research Service compared the inactivation of various drugs in patients with severe Laennec's cirrhosis and in normal subjects. The drugs studied (phenylbutazone, aminopyrine, antipyrine, salicylic acid, and dicumarol) had previously been shown to be metabolized in the body through the action of liver enzymes

The biologic half-life of these drugs was not significantly different in the cirrhotics and controls, indicating that the activity of the enzyme systems involved was not appreciably affected by the liver-damaging disease. Special precautions are usually advised in the administration of certain other drugs to patients with liver disease, for it is commonly believed that drug metabolism is generally impaired in the diseased liver The present studies should be extended to patients with other types of liver disease as well as to other compounds that are metabolized along other similar pathways If indications of the present findings are borne out by further study, some other explanation should

female sex hormones. Previous attempts had been made to separate and analyze steroids by gas chromatography, but had been at best only partly successful. Unfortunately, with ordinary liquid phases and column temperatures, most steroids were so soluble in the liquid phase that they would be retained too long in the column. As a result, many heat-sensitive steroids would decompose before entering the detector.

The scientists overcame these problems with a column whose inert particles were coated with a very thin film of methyl silicone gum. The column could be used at low temperatures and gave rapid transit times, a combination which solved the steroid heat-sensitivity problem. At the same time it provided high sensitivity—being able to analyze samples weighing as little as one millionth of a gram—and could separate components differing only very slightly in their molecular structure.

In addition to offering a unique combination of speed, sensitivity, and high resolution, the column has proved to be versatile as well. It has been used successfully to separate and analyze a large number of alkaloids, an enormous family of plant substances which has yielded many valuable drugs over the years.

The column and its uses were reported at the Milan Symposium on Drugs Affecting Lipid Metabolism and in *The Journal of the American Chemical Society* (*82* 3181, 3791).

Report Gas Chromatography Detector Based on Sound Velocity Variations

An engineer in the NHI Laboratory of Technical Development has designed detecting equipment utilizing measurement of sound velocity in gases for characterizing the constituents of samples separated by gas chromatography.

For maximum usefulness as an analytical tool in the biologic sciences, the principle of gas chromatography as introduced from England has required the design and incorporation of more sensitive and versatile systems for detecting the constituents of complex samples once the chromatograph had separated them.

Investigators in the NHI Laboratory of Technical Development have attacked this problem on several fronts with notable success, designing and developing detecting systems which permit extension of gas chromatography to many new research areas.

New detecting equipment using variations of measured sound velocity was described by Frank W Noble at the Thirteenth Annual Conference on Electrical Techniques in Medicine and Biology held in Washington, D.C. The velocity of sound passing through a gas varies

according to the physical properties of the gas, such as its molecular weight. With the equipment described, voltage is applied to a sound-transmitting crystal at one end of a tube containing the sample, and recorded by a receiving crystal at the other end. Phase differences induced by particular samples between the two voltages are sufficiently definitive to imply analytical usefulness for the principle.

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be sought for the presumed sensitivity to certain drugs of patients with impaired liver function.

These findings were published in *Medicina Experimentalis* (*1: 290-292*).

Reflexes From Carotid Sinus Important in the Control of Heart Output

Reflexes originating in the carotid sinus have been shown by NHI scientists to control the vigor with which the heart's receiving chambers (atria) pump blood into the ventricles This, in turn, importantly affects ventricular performance and cardiac output.

The carotid sinus has long been known to be sensitive to changes in blood pressure and to initiate nervous reflexes to compensate those changes. However, it was formerly thought that this was accomplished primarily by bringing about changes in peripheral resistance to blood flow and by reflexly changing heart rate. Now studies at the NHI Laboratory of Cardiovascular Physiology have revealed that when arterial pressure falls, the carotid sinus, in addition to reflexly increasing peripheral arteriolar and venous tone and heart rate, also increases the force of atrial contraction

This is done by diminishing the activity of the vagus nerve (which normally restrains the atrial pulse) while simultaneously increasing the activity of the cardiac sympathetic nerves Sympathetic stimulation increases the vigor of atrial contraction, presumably by increasing heart levels of catechol amines These increase the excitability of the atrial muscle fibers and also the synchronization with which they contract

The increased vigor of atrial contraction pumps more blood into the ventricle, distending it and elongating its muscle fibers more than usual Since muscle fibers contract more forcefully from longer fiber lengths than from shorter ones (provided that the chemical environment of the fibers is not altered), the result is a more vigorous contraction of the ventricle and hence increased heart output

The opposite takes place when arterial pressure is high The carotid sinus reflexly increases vagus activity while decreasing sympathetic activity This suppresses the atrial pulse, reducing ventricular filling pressure and decreasing heart output

These reflexes, called the carotido-sympatho-atrial and carotido-vago-atrial reflexes, are held to be of substantial importance in the regulation of the circulation in changing states They are particularly important at high heart rates, which give the ventricles less time to relax and the atria less time to fill them adequately between pumping strokes. By causing the atria to deliver more blood to the ventricles more rapidly under these conditions, these reflexes help to maintain the heart output at appropriate levels at high heart rates

The work leading to the discovery of the carotido-atrial reflexes was done by Drs Stanley J. Sarnoff, J. P. Gilmore, and J. H Mitchell. Their findings were reported at the Federation Meetings in Chicago.

Alcohol Causes Deposition of Fat in Liver by Action on Pituitary

NHI scientists have found that large single doses of alcohol in rats interfere with pituitary control over the fat transport system. As a result, excessive amounts of triglycerides (neutral fat) are mobilized from adipose tissue as free fatty acids and carried by the plasma to the liver, where they are recombined with glycerol and deposited in this organ This derangement of fat transport may be important in the development of the cirrhosis often found in alcoholics, in which the chronic deposition of excessive fat in the liver is thought to lead eventually to necrosis

Single orally administered doses of 4.8 grams of alcohol per kilogram of body weight (equivalent in humans to about six double martinis) resulted 18 hours later in a threefold increase in liver triglycerides in female rats Larger doses increased liver triglycerides almost fivefold

That these effects were due to the mobilization of fatty acids from adipose tissue rather than to an increased synthesis of fatty acids by the liver was shown by the linoleic acid content of the deposited fat. This unsaturated fatty acid cannot be synthesized by the rat, but is of dietary origin When assayed by gas chromatography, the liver fat deposited by action of the alcohol was found to have virtually the same linoleic content as adipose tissue Similar results were obtained when the oleic acid content was assayed Since the concentrations of both fatty acids in liver reflect that in adipose tissue, little if any of the fat deposited as a result of the alcohol could have come from fatty acids synthesized by the liver

The finding that alcohol did not produce these effects in rats whose pituitaries had been removed suggested that the effects of alcohol on fat transport were mediated through hormones from this master gland Investigation of the effects of alcohol on the pituitary-adrenal axis indicated that alcohol causes the pituitary to release ACTH and perhaps other hormones important in fat mobilization However, the mechanism by which alcohol does so is not known

The scientists found that pretreating rats with certain adrenergic blocking agents prevented the alcohol-induced fat deposition in liver, suggesting that the catechol amines may also be involved in the process It is not yet clear whether the protection conferred by these agents is due to their blocking the action of amines at nerve endings

which innervate fat cells or to their blocking the release of pituitary hormones. Studies of the mechanism or mechanisms involved are underway in the hope that these might lead to drugs that can prevent or ameliorate Laennec's cirrhosis.

The experiments were conducted by Drs. B. B. Brodie, H M. Maling, W. M. Butler, Jr. and R. P. Maickel, of the Laboratory of Chemical Pharmacology, and by Dr. M. G Horning, of the Laboratory of Cellular Physiology and Metabolism. Their findings were reported at the Symposium on Neurological and Hepatic Complications of Alcoholism held in New York.

Myocardial Edema Result of High Atrial Pressure in Congestive Failure

Studies on the mechanism of myocardial edema—the accumulation of water and electrolytes in the heart muscle that often accompanies congestive heart failure—indicate that the major pathogenic factor is the elevated right atrial pressure also frequently found in this condition Excessive serum levels of aldosterone, a hormone which promotes water and salt retention, and low serum protein levels may also play a role in the process

The studies were performed on 46 dogs—21 normals and 25 with experimentally produced lesions causing chronic congestive heart failure or chronic ascites (accumulation of fluid in the abdominal cavity) After periods ranging from 6 to 189 days, the animals were anesthetized, sacrificed, and samples of heart tissue immediately taken for determination of water, fat and electrolyte content.

The experimental data were then analyzed to evaluate the importance of the following factors in myocardial edema the duration of heart failure, enlargement of the ventricles, serum aldosterone and protein levels, and elevation of right atrial pressure

All of the experimental lesions produced moderate to marked increases in heart muscle content of water, sodium, and chloride The observed increases in myocardial chloride suggested that the edema was the result of accumulation of extracellular fluid

Comparison of data from dogs dying from heart failure within 6 days with the data from another group surviving for 3-6 months ruled out duration of heart failure and ventricular enlargement as significant factors in myocardial edema Other data suggested that high serum aldosterone and low serum protein levels, while not determining factors in themselves, do play supporting roles in the process

The factor that appeared to be most important in myocardial edema was elevation of right atrial pressure The degree of increase correlated well with the severity of the edema Increased right atrial pressure appears to result in passive venous congestion, which, when

combined with high serum aldosterone and low serum protein levels, leads to the accumulation of water, sodium, and chloride in heart muscle that constitutes myocardial edema.

These experiments were performed by Drs. N. A. Yankopoulos, J. O. Davis, Ernest Cotlove, and Mary Trapasso of the Laboratory of Kidney and Electrolyte Metabolism Their findings were reported in the *American Journal of Physiology* (199. 603-608)

Neutral Fat Made in Adipose Tissue by Mechanism Similar to That in Liver

NHI scientists have found that fatty acids are converted to their neutral storage form (triglycerides) in adipose tissue by a mechanism very similar to that reported by others for triglyceride synthesis in liver. The studies also indicate that the release of free fatty acids from adipose tissue may be importantly influenced by factors affecting the rate of triglyceride synthesis

In these studies Drs Daniel Steinberg, Martha Vaughan, and Simeon Margolis, of the Laboratory of Cellular Physiology and Metabolism, and Dr. Arthur Karmen, of the Laboratory of Technical Development, measured the conversion of a radioactive fatty acid (palmitic acid) to triglycerides and other neutral fat in rat adipose tissue. This tissue was ground up and the homogenized extract divided into two portions One portion was then fortified with substances known to favor triglyceride formation, the other portion, unfortified, was used as the control tissue

The incorporation of radioactive palmitic acid into neutral fat was observed only in the fortified tissue and was almost exclusively in the form of diglycerides and triglycerides Knowing what substances had been added to the fortified tissue, the scientists were able to determine the probable sequence of biochemical steps by which triglycerides are formed *in* adipose tissue. This was found to be very similar to the sequence previously reported by others for triglyceride synthesis in liver

Triglycerides, the form in which most fats are stored in the body, are neutral esters resulting from the combination of three fatty acid molecules with one molecule of glycerol. However, under the biochemical conditions of the body, this combination cannot occur directly Instead, the fatty acids must first be combined with coenzyme A, which serves as a "carrier" in the process Two of the combined molecules (fatty acyl-CoA) then react with glycerol, forming a diglyceride and regenerating coenzyme A With coenzyme A again the "carrier," the third fatty acid is added to the diglyceride to form the triglyceride The triglyceride thus formed can in turn be broken down to yield its fatty acids to meet the body's energy requirements during the fasting state

which innervate fat cells or to their blocking the release of pituitary hormones. Studies of the mechanism or mechanisms involved are underway in the hope that these might lead to drugs that can prevent or ameliorate Laennec's cirrhosis.

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The scientists found that doses of reserpine which depleted brain stores of norepinephrine and serotonin, producing marked sedation, also provoked a sustained activation of the pituitary-adrenal system This activation was manifested in rats by: (1) a fall in adrenal ascorbic acid, accompanied by a rise in plasma corticosterone; (2) increased activity of tryptophan peroxidase (TPO), a liver enzyme whose activity is an indicator of pituitary-adrenal stimulation; and (3) increased mobilization of free fatty acids from adipose tissue.

These responses are similar to those produced by the stress of prolonged exposure to cold, and were not produced by reserpine or cold in animals whose pituitaries or adrenals had been removed This provided further evidence that these responses to reserpine result from pituitary-adrenal activation and are mediated by ACTH hypersecretion.

Hypersecretion of ACTH does not result from the action of reserpine *per se*, but is related to its depletion of brain serotonin stores. Doses of reserpine that deplete brain serotonin by less than 50% do not elicit noticeable sedation nor hypersecretion of ACTH; slightly larger doses elicit both responses, which become more marked with increasing doses until a maximum is reached with the complete depletion of brain serotonin stores That ACTH hypersecretion is related specifically to *serotonin depletion* has been shown by experiments using alpha-methyl-m-tyrosine Doses of this amino acid that depleted brain norepinephrine stores with very little effect on brain serotonin elicited neither sedation nor ACTH hypersecretion

This indirect action of reserpine also explains why the "stress" responses, like the drug's sedative effects, persist long after the administered reserpine has disappeared Apparently, reserpine is a "hit-and-run" drug which depletes the brain of amines by inactivating or destroying their storage sites The brain amines remain depleted until the storage sites recover, when brain amines slowly return to normal. The "stress" responses will persist until brain serotonin again reaches 50% of normal

These studies point out the potential value of pharmacological tools in clarifying how the brain directs biochemical mechanisms, and that the interaction of drugs with these mechanisms may be an important aspect of drug action

Rheumatic Fever Studies Reveal Predictability of Heart Damage

NHI grant-aided studies of rheumatic fever recurrences at a rheumatic fever prophylaxis clinic indicate that: (1) signs of heart valve damage in susceptible patients usually appear during the initial attack of rheumatic fever, and (2) patients free from valvular involvement during the first attack usually remain free from rheumatic heart disease despite recurrent attacks

The scientists observed that the release of free fatty acids (FFA) from adipose tissue appeared to be influenced by the rate of triglyceride synthesis. In the fortified tissue, where biochemical conditions favored triglyceride synthesis, there was a net uptake of FFA; however, in the unfortified tissue there was a net release of FFA. A number of hormones are known to stimulate FFA release, and these observations suggested that their actions might be due in part at least to their effects on triglyceride synthesis

In experiments following up this lead, Drs. Steinberg, Vaughan, and Margolis determined the effects of glucose, ACTH, epinephrine, and serotonin on triglyceride synthesis in intact adipose tissue incubated in a medium containing radioactive palmitic acid.

When glucose, an inhibitor of FFA release, was added to the incubation medium, the incorporation of the radioactive palmitic acid into triglycerides was more than doubled in the tissue sample. However, the addition of ACTH or epinephrine, hormones which stimulate FFA release, reduced triglyceride synthesis by 50% or more The reduction in triglyceride synthesis was accompanied by increased FFA concentrations both in the tissue samples and in their incubation media Serotonin, a hormone which does not affect FFA release, also did not affect the rate of triglyceride synthesis These data thus lend support to the hypothesis that hormonal control over FFA release is exerted, at least in part, over the rate of triglyceride synthesis

These findings were reported in *Federation Proceedings* (*19* 227) and in the *Journal of Biological Chemistry* (*235* 38–39).

Tranquilizer Triggers Pituitary-Adrenal "Stress" Responses

Reserpine, a drug in wide clinical use as a hypotensive and tranquilizing agent, has been found to trigger certain body responses usually associated with stress hypersecretion of ACTH, increased serum levels of glucocorticoids from the adrenal cortex, and excessive mobilization of free fatty acids from adipose tissue The paradox implicit in these actions of reserpine is heightened by experimental evidence that these "stress responses" (*1*) are produced only by doses of reserpine sufficiently large to produce overt sedation, and (2) are the indirect result of the same action of the drug believed responsible for its tranquilizing effects—depletion of brain serotonin stores

These findings were made by Drs Bernard B Brodie, Erik O. Westermann, and Roger P Maickel, of the NHI Laboratory of Chemical Pharmacology, as part of a series of animal studies on the role of the hypothalmo-pituitary-adrenocortical system in biochemical adaptation They were presented by Dr. Brodie at the Fourth International Neurochemical Symposium, held at Varenna, Italy

storehouse for these master plans. RNA, located out in the microsomal structures of the cell, is widely regarded as a template, or "die" in which the protein is cast.

Dr. Hendler turned instead to the lipids, which he notes, are even more abundant than RNA in some of the microsomal structures thought to be sites of protein synthesis. (The ribosomes, richest of all in RNA, have been reported to contain about five times as much lipid as RNA)

From studies of synthesis of the egg protein, albumin, by the hen oviduct, Dr. Hendler has gained evidence that the basic amino acid structural units of proteins combine with lipids very early in the protein assembly process to form more complex units (amino acid complexes) So far eleven albumin amino acids have repeatedly been seen to combine with the lipids in these complexes The findings suggest the possibility that the lipid-associated amino acid groups may actually represent primitive precursors of the natural protein molecule.

From the evidence accumulated, Dr Hendler speculates that the basic building blocks of the protein (the amino acids), arriving at the cell via the watery fluids of the body, are made fat soluble, entering into the lipid mechanism very early in the protein assembly process It is in this lipid medium that their first orderly grouping seems to occur—perhaps the first step in the precise forging of the complex links (polypeptides) that will make up the great chains of the finished protein

Dr Hendler is also considering the possibility that the whole assembly process—genetic "master plan" to emergence of the finished molecule—is a lipid process, the study of which might fill major gaps that remain in current understanding of how proteins are made With this as a working hypothesis, he is continuing in his attempts to sift out recognizable protein precursors in new lipid-associated amino acid complexes

He is also hoping to find the chemical process by which the individual amino acids are made fat soluble, for this might be the key step which introduces the raw materials of the protein—the amino acids—into the cell's machinery for making the protein.

Myosin Molecule Consists of Three Tightly Coiled Protein Chains

Recent advances in protein chemistry at NHI and elsewhere permit the conclusion that the myosin molecule is made up of three identical chains, each wound in a tight coil and all three twisted together as a long rope

The protein myosin is of interest in terms of cardiovascular functions because it is believed to be the predominant component of the contractile system of muscle A program of research on myosin at NHI has the

These findings were reported in *The New England Journal of Medicine* (262: 533–540) by Drs. Alvan R. Feinstein and Mario Spagnuolo of Irvington House, Irvington-on-Hudson, New York, and the New York University College of Medicine in New York City.

Their results are based on analyses of 370 documented attacks of rheumatic fever in 161 patients at Irvington House. Only those patients whose attacks fulfilled the modified Jones criteria for the diagnosis of rheumatic fever were included in the study.

The New York scientists found that valvulitis, as determined by careful stethoscopic examination, was a feature of the initial attack in 90 of these 161 patients. With subsequent attacks, the valvulitis remained unchanged or became worse and in some cases was accompanied by cardiac enlargement, pericarditis, or congestive heart failure.

Valvular involvement was not found in the initial rheumatic episodes of 71 patients. With subsequent attacks, all but 10 of these patients remained free of valvulitis and other symptoms of heart disease. Although 10 patients were found to have a significant diastolic murmur at a subsequent attack, the investigators speculate that in at least 8 of these 10 patients, the murmur was present but not recognized at the first attack

"The results suggest that the rheumatic host has a specific and persistent susceptibility to get or to avoid valvular involvement If it does not occur with the first attack, it does not occur (with dubious exceptions) in the subsequent attacks. In most cases in which rheumatic heart disease develops, the valvular involvement is manifested in the very first attack," conclude Drs Feinstein and Spagnuolo

Their findings, which should be of considerable interest to the parents of rheumatic children, may enable a reduction in the duration of rheumatic fever prophylaxis for valvulitis-free patients However, "Prophylaxis should not be stopped in this group without careful auscultatory confirmation of cardiac status and until further studies confirm the present conclusions," state the scientists

Lipids Play Major Role in Protein Synthesis, NIH Studies Suggest

Evidence for a major role of intracellular lipids in protein synthesis has been gained from studies by Dr Richard Hendler of the NIH Laboratory of Cellular Physiology and Metabolism He reported these findings at the Federation Meetings

In the search for the intermediate through which the stored genetic master plans for each protein molds and binds its amino acid structural units into the finished molecule, protein chemists in many countries are concentrating on variants of the nucleic acids, DNA and RNA Genetic DNA, in the nucleus of the cell, is widely regarded as the

taining albumin and glucose, Dr. Vaughan found that hormonal stimulation of UFA release was accompanied by increased uptake of glucose by the tissue and by stepped up phosphorylase activity.

Phosphorylase is an enzyme which breaks down glycogen, the form in which carbohydrates are stored in the body, to yield glucose, an important energy source. In the liver, the resulting glucose can be released into the blood by action of the enzyme phosphatase However, the phosphatase is not present in adipose tissue, so that the small amounts of glucose resulting from phosphorylase activity must be used locally, either as a source of energy or for conversion into fatty acids and triglycerides

The fact that all four hormones stepped up phosphorylase activity at the same time that they stimulated UFA release suggested that the hormones might control UFA release by a common mechanism. Phosphorylase is not the common denominator, however, since previous work at this laboratory had shown that serotonin, a hormone which increased phosphorylase activity, did not stimulate UFA release.

But the increased phosphorylase activity produced by ACTH in adrenal tissue and by epinephrine and glucagon in liver does have a common denominator--cyclic 3,5 adenylic monophosphoric acid. The hormones have been shown by others to stimulate the accumulation of this compound, which in turn increases active phosphorylase It is probable that the compound may play a similar role in adipose tissue in the intact animal, even though the compound produced no demonstrable effects on phosphorylase in the *in vitro* studies

It is also possible that 3,5 AMP may play a role in hormone-stimulated UFA release, perhaps by affecting reactions in addition to that producing increased phosphorylase

These findings were reported at the meeting of the American Society of Biological Chemists.

Active Ion Transport in Red Cell Ghost Powered by ATP

Studies by Dr Joseph Hoffman, of the NHI Laboratory of Kidney and Electrolyte Metabolism, have shown that adenosine triphosphate, a compound that powers many of the body's energy-consuming processes, is also the direct energy source for active ion transport in the red blood cell His findings were reported at the Federation Meetings.

Active ion transport is the process whereby the cell can actively and selectively transport electrically charged substances (ions) across the cell membrane against opposing electrochemical forces In this process, the "ion pump" maintains normal electrolyte balance within the cell by using ATP generated by cell metabolism to offset the loss of ions across the cell membrane by passive diffusion

immediate objective of interpreting some of the properties of myosin on the basis of its molecular structure, and is ultimately aimed at understanding its role in the contraction of muscle.

Like other proteins the myosin molecule is made of amino acid groups, bound together as links in the long chains which constitute its primary structure Although myosin is known to be a comparatively long slender molecule (1600 x 22 Angstrom units), it has not been known how many polypeptide chains are held together by secondary bonds to form the molecule and, if more than one chain, whether or not they differ from one another.

Drs. W. Wayne Kielley and William F. Harrington, in the NHI Laboratory of Cellular Physiology, have approached this problem using guanidine hydrochloride to break the secondary bonds holding the chains together, while leaving intact the primary bonds holding together the polypeptide links of each chain.

Examining the products of this dissociating process by sedimentation in the ultracentrifuge, together with observations of their diffusion rates, viscosities, optical rotation properties, and other data, the investigators have concluded that the myosin molecule consists of three identical polypeptide chains, each wound into a tight helical coil, the three chains themselves being wound together in the form of a long rope.

These findings were published in *Biochimica et Biophysica Acta* (41: 401–421).

Nature of Hormone Control Over Fatty Acid Release Explored by NHI Studies

Recent studies at the National Heart Institute have explored the mechanisms whereby fatty acids are stored in the body's fat depots as triglycerides (neutral fat) or are released from these depots as unesterified fatty acids (UFA) to meet the body's energy requirements The studies sought to clarify the mechanism or mechanisms by which four hormones—epinephrine, norepinephrine, ACTH, and glucagon—stimulate UFA release from adipose tissue

Triglycerides, the form in which most fats are stored in the body, are neutral esters formed by the combination of three molecules of fatty acid with one molecule of glycerol The triglyceride can in turn be broken down to yield three molecules of UFA, an important metabolic fuel during the fasting state The latter process is known to be subject to a number of hormonal controls.

Seeking to clarify the nature of these hormonal controls, Dr. Martha Vaughan, of the Laboratory of Cellular Physiology and Metabolism, studied the effects on adipose tissue of four hormones shown by others to stimulate the release of UFA epinephrine, norepinephrine, ACTH, and glucagon Using rat adipose tissue incubated in a medium con-

Presumably because one ion cannot replace the metabolic functions of another, the cell must maintain the proper concentrations of the proper ions both for the regulation of its volume and for its metabolic operations. This would be impossible but for the delicate balance struck between passive loss on the one hand and active transport on the other.

Previous work on intact red blood cells by Dr. E. T. Dunham, formerly of the same laboratory, had indicated that ATP might be the fuel for the ion pump. In studies following this lead, Dr. Hoffman used red cell "ghosts" whose normal contents had been considerably reduced by hemolysis. The ghosts not only retained the membrane characteristics of the intact cell, but could also be reconstituted containing known substances whose metabolism could subsequently be observed without the interference that might result from other metabolites and metabolic processes in the intact cell.

Dr. Hoffman's studies showed that ATP was the specific fuel for the ion pump in the ghost system; however, if no ATP was incorporated into the ghost, the pump would still work (though less efficiently) if other substances that would generate ATP were incorporated. These findings suggest that the ion pump requires the presence of an enzyme (an ATPase) in order to use ATP. Such an enzyme has been characterized in membranes isolated from red blood cells by Dr. R. L. Post of Vanderbilt, and appears to possess the same activation characteristics as does active ion transport in both red cells and other ghosts. Thus the evidence is strong that the ion pump either *is* an ATPase, or else has an ATPase as an intimate component.

Protein and Fat Fractions of Lipoprotein Molecule Provided by Liver

Scientists at NHI have found that the liver, previously shown to be the source of the lipid portion of lipoproteins, is also the source of the protein of this complex molecule, the form in which fats are transported in the blood. Experiments conducted by Drs. Charles Radding and Daniel Steinberg, of the Laboratory of Cellular Physiology and Metabolism, have demonstrated that rat liver slices, incubated *in vitro*, synthesize and secrete high-density lipoproteins identical with those found in normal rat serum. Their findings are reported in *The Journal of Clinical Investigation*.

The liver slices were incubated in rat serum with a complete mixture of amino acids labeled with carbon-14. After incubation periods ranging up to four hours, the slices were removed and samples of the media ultracentrifuged to separate the lipoprotein fractions. These were then analyzed by the "fingerprint" technique.

When broken down by the proteolytic enzymes trypsin or chymo-

trypsin, each protein yields its own characteristic peptide degradation products. These can be spotted on filter paper and separated on the vertical axis by descending paper chromatography, then on the horizontal axis by electrophoresis to form a characteristic peptide pattern. When this pattern is developed by ninhydrin staining, the result is a distinctive "fingerprint" which identifies the protein.

The ninhydrin fingerprints of high-density lipoproteins from the incubation medium were compared with fingerprints of lipoproteins of the same density class from normal rat serum and found to be identical. Autoradiograms (made by placing the media fingerprint strips in contact with X-ray film for 1–2 months, then developing the film) showed the presence of radioactivity in all of the ninhydrin spots of the fingerprint.

These comparisons showed that the lipoproteins synthesized by the liver slices were identical to those of normal serum, and, since labeled amino acids had been incorporated into all of the newly synthesized lipoproteins, that the liver was the source of the protein as well as the lipid portions of those lipoproteins

To determine whether the rate of lipoprotein synthesis is affected by the rate of cholesterol synthesis, the scientists pretreated rats to speed up or slow down their cholesterol production Liver slices from these rats were then incubated with acetate (a cholesterol precursor) and an amino acid, both tagged with radioactivity so that the rates of cholesterol and lipoprotein synthesis could be measured simultaneously

Lipoprotein synthesis was not noticeably affected even by wide variations in the rate of cholesterol synthesis in these experiments, which suggested that the two rates were independent of one another This was not proved conclusively, however, because of the possibility that enough cholesterol might already have been formed in the tissue before incubation was begun to support normal rates of lipoprotein synthesis during the short period of experimental observation, but not over extended periods

Other experiments showed that liver slices from nephrotic rats synthesized both lipoproteins and other serum proteins more rapidly than did those from normal rats This suggests that overproduction of lipoproteins may be an important factor in the hyperlipemia of nephrosis

Observe Accelerated Drug Metabolism in Pretreated Rats

Pretreating rats with a variety of drugs has been found by NIH scientists to increase the activity of drug-metabolizing enzyme systems in the liver, thus decreasing the duration of drug action in the body

The findings were reported in the *Federation Proceedings* (19 390) by

Presumably because one ion cannot replace the metabolic functions of another, the cell must maintain the proper concentrations of the proper ions both for the regulation of its volume and for its metabolic operations. This would be impossible but for the delicate balance struck between passive loss on the one hand and active transport on the other.

Previous work on intact red blood cells by Dr. E. T. Dunham, formerly of the same laboratory, had indicated that ATP might be the fuel for the ion pump. In studies following this lead, Dr. Hoffman used red cell "ghosts" whose normal contents had been considerably reduced by hemolysis. The ghosts not only retained the membrane characteristics of the intact cell, but could also be reconstituted containing known substances whose metabolism could subsequently be observed without the interference that might result from other metabolites and metabolic processes in the intact cell.

Dr. Hoffman's studies showed that ATP was the specific fuel for the ion pump in the ghost system, however, if no ATP was incorporated into the ghost, the pump would still work (though less efficiently) if other substances that would generate ATP were incorporated. These findings suggest that the ion pump requires the presence of an enzyme (an ATPase) in order to use ATP. Such an enzyme has been characterized in membranes isolated from red blood cells by Dr. R. L. Post of Vanderbilt, and appears to possess the same activation characteristics as does active ion transport in both red cells and other ghosts. Thus the evidence is strong that the ion pump either *is* an ATPase, or else has an ATPase as an intimate component.

Protein and Fat Fractions of Lipoprotein Molecule Provided by Liver

Scientists at NHI have found that the liver, previously shown to be the source of the lipid portion of lipoproteins, is also the source of the protein of this complex molecule, the form in which fats are transported in the blood Experiments conducted by Drs Charles Radding and Daniel Steinberg, of the Laboratory of Cellular Physiology and Metabolism, have demonstrated that rat liver slices, incubated *in vitro*, synthesize and secrete high-density lipoproteins identical with those found in normal rat serum. Their findings are reported in *The Journal of Clinical Investigation*

The liver slices were incubated in rat serum with a complete mixture of amino acids labeled with carbon-14 After incubation periods ranging up to four hours, the slices were removed and samples of the media ultracentrifuged to separate the lipoprotein fractions. These were then analyzed by the "fingerprint" technique.

When broken down by the proteolytic enzymes trypsin or chymo-

acid (aspirin) in preventing residual rheumatic heart disease. The NHI grant-aided studies were conducted by the Combined Rheumatic Fever Study Group which consists of 12 investigators in Children's Cardiac Services of 8 hospitals. 4 in New York City, 2 in Baltimore, and 1 each in Boston and Cleveland. The investigators, coordinated by Dr. A. G. Kuttner of New York University-Bellevue Medical Center, report their combined findings in the *New England Journal of Medicine* (262: 895–902).

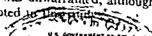
Their findings are similar to those of an earlier cooperative study of aspirin vs. steroid therapy, sponsored jointly by the Research Council of Great Britain and the American Heart Association. In the present study, however, steroids were given in larger doses and over a longer period of time.

Admitted to the study were 57 patients who met the following criteria. (1) they were 12 years of age or younger, (2) had suffered their first attack of rheumatic fever not more than 28 days previously, and (3) had moderate to severe carditis as evidenced by pericardial rub or effusion, cardiac enlargement, congestive heart failure, and/or significant heart murmurs. About half of these patients received large daily doses of prednisone which totalled 3 grams over a 12-week treatment period, the remaining patients were given doses of acetylsalicylic acid sufficient to maintain blood levels at 25 to 35 mg./100 cc., also for a 12-week period. Three weeks after termination of therapy and again 1 year later all patients were re-examined and the incidence of residual heart disease in each group compared.

The study group found that both prednisone and aspirin controlled acute rheumatic symptoms in most patients, and, during the ensuing year, all patients remained free of rheumatic recurrences. Of those patients available for study at the end of the year, 12 in the prednisone group and 16 in the aspirin group recovered completely with no signs of residual rheumatic heart disease, whereas 16 prednisone and 7 aspirin-treated patients had residual heart disease at this time. Of 3 patients originally allocated to the aspirin group but later switched to prednisone, 1 recovered completely and 2 were left with residual heart disease.

Thus, "large doses of prednisone given for 12 weeks were not found to be superior to acetylsalicylic acid in preventing residual rheumatic heart disease," conclude the investigators. "The chief action of both prednisone and salicylates appears to be to suppress the inflammatory reaction caused by this disease. In many patients, however, even if the acute symptoms are promptly and well controlled, cardiac damage is not prevented," they state.

In view of these findings, all but one of the investigators felt the risk of prolonged steroid therapy was unwarranted, although no serious side effects of prednisone were noted [illegible]



U.S. GOVERNMENT PRINTING OFFICE: 1961 OF—581031

Drs. A. H. Conney, I. A. Michaelson, and J. J. Burns of the NHI's Laboratory of Chemical Pharmacology. This laboratory is conducting a broad program of basic studies aimed at elucidating the factors regulating duration of drug action. Previous studies in this laboratory had shown that special enzyme systems in liver microsomes, tiny particles in liver cells, inactivate many drugs as well as other compounds foreign to the body by converting them into compounds which the kidney can excrete.

In the currently reported studies, Dr. Conney and co-workers investigated the ability of microsomal enzyme systems in rat livers to metabolize several commonly used drugs following repeated doses of the same or another drug Rats were pretreated for four days and their livers incubated with test drugs to determine microsomal enzyme activity. The scientists also tested enzyme activity in live, pretreated rats by measuring the duration of action of test drugs Drugs used in the study included barbiturates, muscle relaxing drugs, analgesics, antirheumatic agents, a carcinogenic hydrocarbon, and an antihistamine.

Results of the liver incubation studies showed that pretreatment with phenobarbital, barbital, phenylbutazone, orphanedrine, or aminopyrine caused "several-fold increases" in the ability of liver microsomes to metabolize hexobarbital, zoxazolamine, phenylbutazone, and aminopyrine, report the investigators They obtained evidence of accelerated drug metabolism in living, pretreated rats by demonstrating a shortened duration of action of hexobarbital, zoxazolamine, meprobamate, and carisoprodol in animals that had received repeated doses of these drugs.

In other *in vitro* studies, the investigators observed that pretreatment with the hydrocarbon, 3,4-benzpyrene, stimulated the microsomal enzyme system that metabolizes zoxazolamine, but not the hexobarbital-metabolizing system. "In accord with this, 3,4-benzpyrene administration shortens the duration of zoxazolamine paralysis from 730 minutes to 17 minutes but does not shorten the duration of hexobarbital hypnosis," they report Interestingly, the duration of action of both zoxazolamine and hexobarbital was shortened in rats pretreated with the antihistaminic drug, chlorcyclizine

"The findings that pretreatment of animals with one drug can speed up the metabolism of the same or another drug may be of considerable importance when evaluating the pharmacological activity of drugs that are given repeatedly, either alone or in combination," stated the Heart Institute scientists

Prednisone-Aspirin Equally Effective in Preventing Rheumatic Heart Damage

Cooperative clinical studies have shown that large doses of the steroid, prednisone, are not superior to therapeutic doses of acetylsalicylic

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